


**New Claims:**

Added claims 46-65 are believed to meet patentability standards. As discussed, the combination of steps and techniques employed by applicants has resulted in cryopreserved oocytes that maintain morphology and viability when thawed. In contrast to attempts by others to obtain reasonable numbers of viable oocytes after freezing and thawing, applicants recover at least 86% of viable oocytes. Others using similar but different procedures report, at best, in the range of 30%. Accordingly, a population of cryopreserved oocytes as claimed by applicants is a significant improvement. Such a population is not a "natural form" of oocytes because the majority of wild type oocytes in a stock solution are damaged when cryopreserved.

Applicants intend to have fully addressed any concerns that may have arisen or /might be raised with respect to new claims 46-65. This paper is intended to be a complete response to the examiner's action. Should the examiner have any questions or suggestions, the undersigned respectfully requests a telephone conference at 203.353.6848.

Respectfully Submitted,

  
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**LISTING OF THE CLAIMS**

Claims 36-45 (canceled).

46. (new) A method for cryopreservation of oocytes, comprising:
- suspending oocytes in an equilibration medium;
- rinsing the oocytes in a vitrification solution;
- vitrifying the oocyte suspension by dropping microdroplets of said oocyte suspension onto a solid surface that has a temperature between  $-150^{\circ}\text{C}$  and  $-180^{\circ}\text{C}$ ; and
- collecting frozen microdroplets that contain vitrified oocytes
- wherein said oocytes maintain viability and morphology after thawing.
47. (new) The method of claim 1 wherein the suspending is for a period of about 12-15 minutes at near physiological temperature of  $39^{\circ}\text{C}$ ;
48. (new) The method of claim 1 wherein the rinsing is for about 25-30 seconds.
49. (new) The method of claim 1 wherein the vitrification solution comprises an intracellular cryoprotectant; a macromolecule; a sugar; and a surfactant
50. (new) The method of claim 1 wherein the equilibration medium consists of an intracellular cryoprotective agent, and a macromolecule with surfactant effect at room temperature.
50. (new) The method of claim 49 or 50 wherein the intracellular cryoprotectant is ethylene glycol
51. (new) The method of claim 49 or 50 wherein the macromolecule is polyvinylpyrrolidone.
52. (new) The method of claim 49 wherein the sugar is trehalose.
53. (new) The method of claim 49 or 50 wherein the surfactant is bovine serum albumin, or fetal bovine serum.
54. (new) The method of claim 50 wherein the a base medium is TCM 199.

- 55. (new) The method of claim 1 further comprising storing the collected frozen microdroplets for at least three weeks prior to thawing.
- 56. (new) The method of claim 1 further comprising partially or fully removing oocyte cumulus cells prior to equilibration with the equilibration medium.
- 57. (new) The method of claim 1 wherein the cryoprotective agent raises the vitreous state glass transition temperature in the microdroplets sufficiently to inhibit ice formation.
- 58. (new) The method of claim 1 further comprising thawing the vitrified oocyte microdroplets at a near physiological temperature of about 39°C for up to about 3 minutes.
- 59. (new) The method of claim 9 wherein the oocytes are non-human mammalian oocytes.
- 60. (new) The method of claim 10 further comprising fertilizing the thawed oocyte.
- 61. (new) The method of claim 11 wherein the fertilized thawed oocyte is incubated to form an embryo.
- 62. (new) The method of claim 12 wherein incubation is by cumulus cell-coculture with KSOM and fetal bovine serum media.
- 63. (new) The method of claim 1 wherein the microdroplets have a volume of about 1-10 microliters.
- 64. (new) The method of claim 14 wherein the microdroplets have a volume of 1 microliter.
- 65. (new) A population of morphologically intact viable cryopreserved oocytes prepared by the method of claim 1.